XXXIII. THE ANTISCORBUTIC VALUE OF CABBAGE. I. THE ANTISCORBUTIC AND GROWTH PROMOTING PROPERTIES OF RAW AND HEATED CABBAGE.

By ELLEN MARION DELF,

WITH AN APPENDIX

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ON THE HISTOLOGICAL DIAGNOSIS OF EXPERIMENTAL SCURVY.

From the Lister Institute of Preventive Medicine.

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The antiscorbutic properties of fresh vegetables are well known and cabbages of all kinds are especially valuable in this respect. At the present time, owing to the scarcity of fresh fruit, it is important that the best use should be made of any available green vegetables. Most vegetables require cooking in order to make them digestible and palatable enough for human consumption. It is known, however, that the antiscorbutic value of both vegetables and fruit is diminished by cooking, and the present investigation is an attempt to estimate the loss in the antiscorbutic properties of fresh cabbage after exposure to heat at various temperatures and for different intervals of time.

In order to have some basis of comparison it has been necessary to make preliminary experiments using varying amounts of fresh raw cabbage as the sole antiscorbutic in an otherwise adequate diet. Guinea-pigs were chosen as experimental animals and the standard diet selected was a mixture of rolled oats and bran *ad libitum*, and water. With small rations of cabbage (5 g. daily and under) 60 cc. of milk previously heated in an autoclave for one hour at 120° C. was also given daily. This was found to improve the nutritive value of the diet and encourage growth without influencing the onset of scurvy.

Fig. 1. No. 377. Normal after 3 months on diet of oats, bran, water, and 15 g. cabbage autoclaved 1 hr at 120°

Fig. 2. No. 846. Acute scurvy, showing "faceache position" and also position of back limbs (on diet of boiled germinating lentils, unpublished experiment).

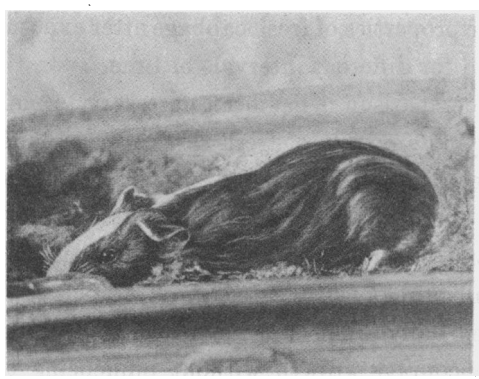

Fig. 3. No. 394. Acute scurvy showing "faceache position" after 20 days, on diet of 5 g. citric acid cabbage steamed 1 hr at 90°, oats, bran and autoclaved milk.

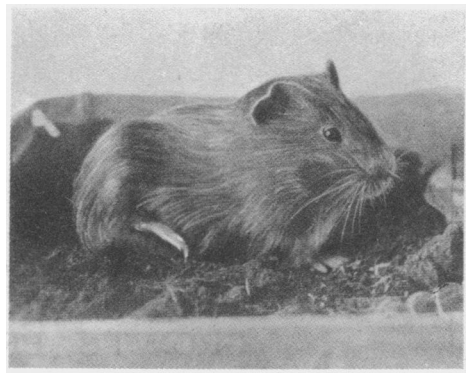

Fig. 4. No. 851. Acute scurvy showing "scurvy position" (foot) (on diet of boiled germinating lentils, unpublished experiment).

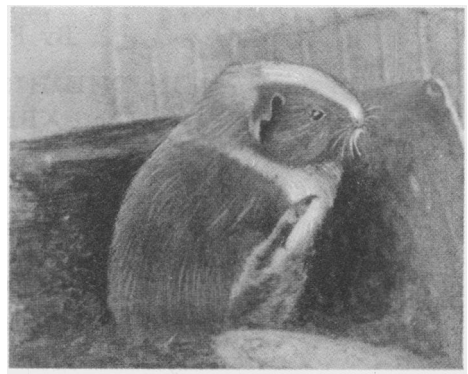

Fig. 5. Acute scurvy in animal on purely scorbutic diet of oats, bran and autoclaved milk, showing nearly vertical position of foot.

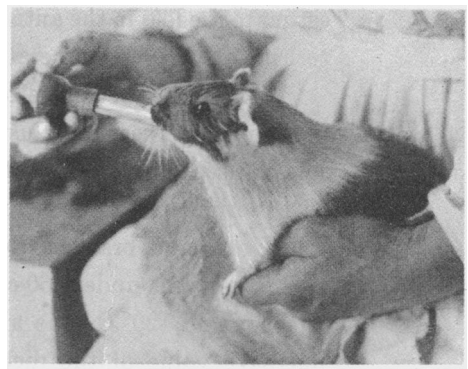

Fig. 6. No. 510. Showing method of feeding autoclaved milk to the experimental animals.

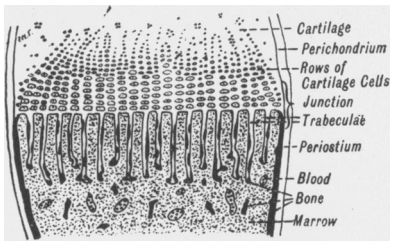

Fig. 1. Normal Rib Junction.

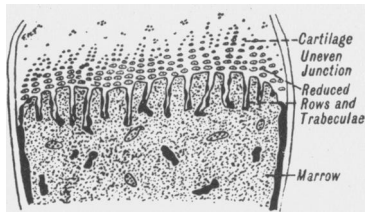

Fig. 2. Nearly Normal Rib Junction, "Incipient Scurvy."

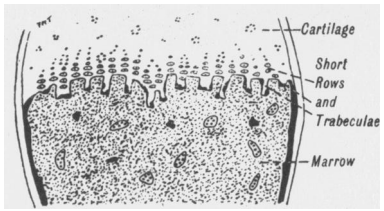

Fig. 3. "Definite Scurvy."

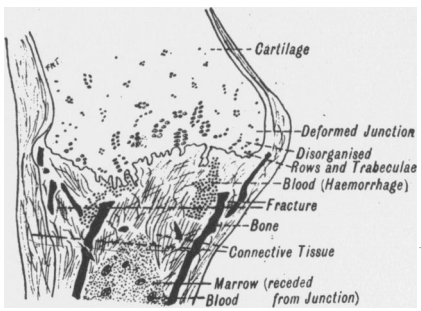

Fig. 4. "Acute Scurvy."

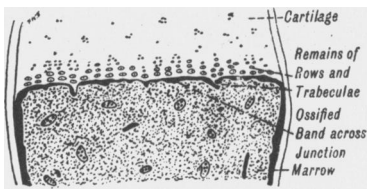

Fig. 5. "Chronic Scurvy" (definite).

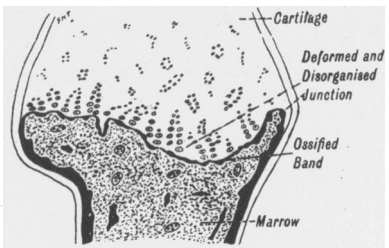

Fig. 6. "Chronic Scurvy" (acute).

The Antiscorbutic Value of Raw Fresh Cabbage.

Experiments of Holst and Frölich [1912] indicate that normal growth of young guinea-pigs can be secured on a diet of fresh cabbage leaves and some kind of cereal food (oats, rice, rye bread or white bread). The change in body-weight relative to the initial weight varied considerably with the amount of cabbage given (see Table I). The only ration which gave both a fairly satisfactory growth and complete protection from scurvy was one of 30 g. daily, but probably younger animals would have made growth on a smaller ration. However, the animals were evidently not under very favourable circumstances, for out of 56 cases cited [Holst and Frölich, 1912, Table IV A], 27 animals died from illness—sometimes of an unknown character, but often from pneumonia or enteritis. Nearly complete protection from scurvy was given by a daily ration of only 1 g., but the animals survived for only a short time with much loss of weight, and in the further work of these authors on dried and heated vegetables, 30 g. of fresh cabbage daily was chosen as the standard ration.

Table I. *Summary of Results of experiments of Holst and Frölich with fresh cabbage (calculated from their paper* [1912, Table IV A, p. 64, and pp. 106–112]).

Ration given	Mean initial weight of animals in g.	No. of animals	Time of experiment in days	Total gain or loss in weight %	Result
30 g. white cabbage and oats and bran *ad lib.*	328	5	100–200	+33	No scurvy
15 g. white cabbage and white bread	507	3	33–105	−25	do.
10 g. white cabbage and white bread	475	4	46–113	−22	Traces of scurvy in one case
10 g. white cabbage and rice (husked)	676	4	28–72	−41	do. do.
25 g. white cabbage and oats and bran *ad lib.*	342	2	15–28	−50	No symptoms in one case, loose teeth in one case
1 g. white cabbage and oats and bran *ad lib.*	307	3	47–56	−50	No symptoms in one case, loose teeth in one case

In our experiments, care has been taken to give the animals the best conditions possible. The laboratory in which they were kept was well ventilated and warmed during the winter (60°–65° F.). Most of the animals were bred on the premises and were well fed and housed from the first. Shortly before starting on an experiment, the animals were placed in separate cages on a litter of peat and were kept under observation for a few days. If the

general condition and the weight curve were satisfactory, the experiment was started when a suitable weight had been attained (about 330–350 g.) but doubtful animals were rejected for the purposes of these experiments.

In the case of animals under experiment, all the rations were given by myself or by fully trained assistants and a daily record was kept of the amount actually consumed. It was generally possible to ensure that the whole of the cabbage ration was eaten, hand feeding being introduced where necessary. All the cabbage was given as green leaf, avoiding both the thickest part of the midrib, and the whitish inner leaves of the heart. In this respect the cabbage used differs from that given by Holst and Frölich who used "white" cabbage (*i.e.* the winter cabbages with large, compact, white hearts) and did not apparently select definitely either the inside or outer green leaves. In our experiments with small rations, especial care was exercised in selecting only the healthy green parts of the turgid leaf to ensure uniformity as far as possible.

Clean water or milk, as the case might be, was given twice daily, and almost every day the animals were allowed to run about on bench or table for exercise. We believe that this regular exercise, even when only for brief periods of time, has a very beneficial effect on their general health and well being. It also allows observation of any inactivity or lameness which is a frequent precursor of the onset of scurvy. For the most part the animals were remarkably healthy; out of the 27 animals on fresh cabbage quoted in Table II only one died from an unknown cause before the completion of the three months of the experiment. In spite of every precaution, however, and the daily disinfection of all the vessels used for feeding, some unexplained deaths occurred occasionally, especially on the smaller limiting ration of cooked cabbage. This may be caused by lowered resistance to chance infection resulting from an insufficient margin of antiscorbutic in the diet to ensure perfect robustness.

Animals of about 330 g. initial weight have been selected as far as possible and the usual duration of an experiment was 90 days. The cereal food given throughout was a mixture of rolled oats and bran which was readily and often greedily eaten. The animals were weighed three times weekly and carefully examined every 5 or 6 days, or oftener when the onset of scurvy was expected.

The symptoms to be found in scorbutic animals have been described in a communication by Chick, Hume and Skelton [1918], but may be recapitulated here. They are: (1) Soreness of joints. (2) Lameness,

with or without general inactivity. (3) The adoption of various typical "scurvy positions" when in repose. In these positions, the limb affected is often held up and in the "face-ache" scurvy position the head is lowered and the neck stretched out (see Plate I, figs. 2 and 3). Post-mortem examination of animals which have frequently adopted the "face-ache" position shows that the teeth are loose and the jaws so fragile as to break with the slightest pressure. (4) Severe cases of long standing have swollen knees often fractured at the epiphysial junction of the tibia, the back limbs being then almost helpless. Photographs of some typical scurvy positions are shown in Plate I.

The post-mortem signs of scurvy are well known and comprise chiefly (1) haemorrhages in the subcutaneous tissue, muscles, periosteum or viscera, (2) fragility of bones, with fracture in severe cases, (3) ridges or swellings on the rib-junctions, (4) loosened teeth set in a fragile jaw and (5) wasted tissues. A systematic examination has been made by Miss F. M. Tozer of the minute structure of the rib-junctions of these and many other experimental animals. On the whole, her independent histological diagnosis falls into line with the symptoms observed during life and with the results of post-mortem examination. In general, it has been possible to distinguish histologically three principal stages in the course of the disease, which may be termed respectively incipient, definite and acute[1]. The incipient stage involves only slight histological abnormality (Plate II, fig. 2) corresponding to absence of definite symptoms in life and very slight evidence of scurvy or none at all at the post-mortem examination. Definite and acute scurvy implies marked disorganisation of the normal structure of the bone cartilage junction (in which growth appears to become arrested in chronic cases) and agrees usually with marked evidence of scurvy during life and at the post-mortem examination. Further details of these stages are given by Miss Tozer in Appendix I together with diagrammatic illustrations (Plate II, figs. 1–6).

Table II gives a summary of the results of the experiments with raw cabbage rations, fuller particulars being given in Table VII. With a 30 g. ration (Exp. 1) the amount of growth was much greater than that found by Holst and Frölich, although the average initial weights of the animals were very similar. The mean growth curve A (Fig. 1) obtained by averaging the body-weights from time to time of all the animals in Exp. 1 has been adopted as the standard of normal growth throughout this investigation. It is nearly identical with the mean curve of normal growth deduced by Chick, Hume and Skelton from the growth of twelve animals upon full

[1] See note, p. 446.

TABLE II. *Experiments with limiting rations of fresh cabbage* (see also Table VII).

Exp.	Ration of fresh cabbage g.	Number of animals	Mean initial weight g.	Total gain %	Time of exp. days	Symptoms in life	Post-mortem examination	Histology of rib-junctions	General conclusions
1	30	3	340	91	87–90	None	Not examined	Not examined	Complete protection
2	15	4	345	74	90	do.	No indications of scurvy	Normal	do. do.
3	5	3	323	26	90	Slight soreness	do. do.	Incipient scurvy	Protection
4	5	3	325	69	90	do.	do. do.	do.	do.
5	2·5	5	323	49	90	Soreness in 2 cases	Brittle leg bones in one case, brittle jaw in another	do.	do.
6	1·5	6	346	49	70–90	Soreness in 5 cases out of 6	Brittle bones in 2 cases	do.	do.
6 A	1·5 (citric acid)	4	330	70	90	Slight soreness after 49 days	2 normal, 2 slight symptoms	1 normal, 3 nearly normal	do.
7	0·5	4	331	18	67–90	Soreness, swollen joints	Three with fragile bones. All with rib-junctions ridged	1 normal 1 slight scurvy 1 chronic scurvy 1 severe scurvy	Scurvy in 3 out of 4 cases

In Expts. 1, 2, and 3 the rest of the ration consisted of water and rolled oats and bran *ad libitum*, the amount consumed daily being about 30 to 60 g. (see Table VII).

In Expts. 4–7 60 cc. milk autoclaved at 120° C. for 1 hour was substituted for the water.

normal diet. The average increase in body-weight is strikingly different in Expts. 1, 2, and 3, diminishing with the reduction of the cabbage ration, although the appetite was not impaired, and the grain consumed was not less (cp. Table VII and curves A, B and C, Fig. 1). In each case there was no symptom of scurvy during life, but the slight tenderness observed in the joints of animals fed on the 5 g. ration and the results of histological examination of their rib-junctions suggest a condition of incipient scurvy.

Since in these animals, growth was apparently limited by the extent of the cabbage ration apart from the incidence of scurvy, another series of animals were given a daily allowance of 60 cc. milk previously autoclaved for an hour at 120°, in order to improve the nutritive value of the diet. Previous experience of workers in this Institute has proved that the addition of this amount of autoclaved milk does not affect the onset of scurvy. Expts. 3 and 4 with a ration of 5 g. cabbage with and without milk, respectively, fully confirm this result. In both sets of animals a departure from the normal could be detected at histological examination of the rib-junctions, though in both sets the animals gave no marked signs of scurvy during life or at the post-mortem examination. The animals on the milk ration however grew much better and were heavier and in better condition throughout the experiment, which lasted three months.

No indications of scurvy were detected during life in most of the animals on 2·5 and 1·5 g. rations of cabbage to which autoclaved milk had been added (Expts. 5 and 6). The mean growth of these animals was less than that of those on the larger rations and the irregular growth on the smaller of these rations is probably significant (C and D, Fig. 2). On histological examination of the rib-junctions incipient scurvy was diagnosed in several cases. On the smallest ration of cabbage given, 0·5 g. (Exp. 7) no growth was made after the first 20 days (E, Fig. 2); three out of the four animals exhibited definite symptoms of scurvy and one died of acute scurvy after 67 days. There is little doubt that most young guinea-pigs would find this ration insufficient for maintenance of normal health. The limiting daily ration of fresh cabbage just protecting a young guinea-pig from definite symptoms of scurvy during life and maintaining health, upon the standard diet of oats and bran and autoclaved milk must therefore lie between 1·5 and 0·5 g. In the experiments of Holst and Frölich a ration of 1 g. fresh cabbage daily was found to give nearly complete protection from scurvy (Table I), and in the present communication this ration has been regarded as the probable minimum protective ration.

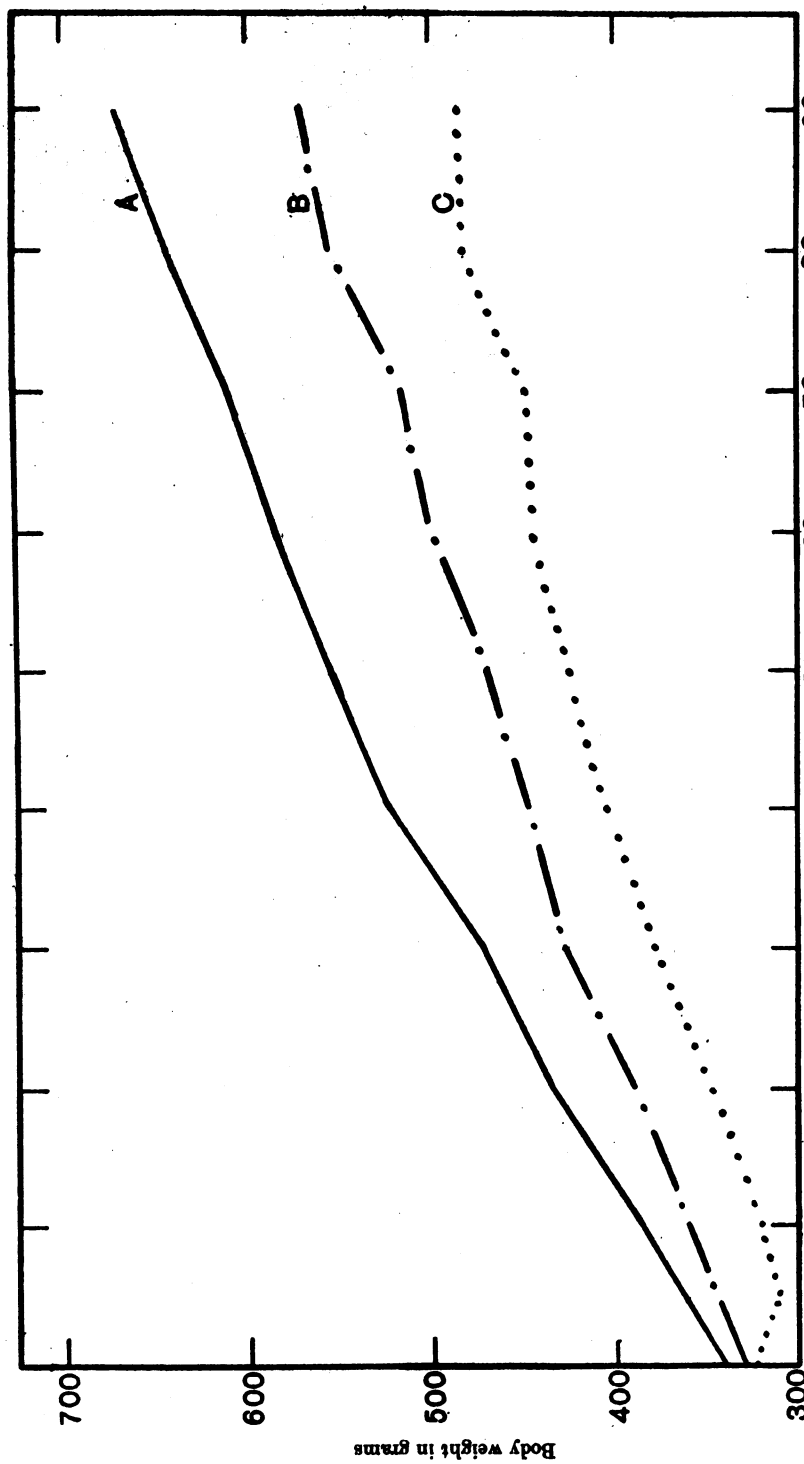

Fig. 1. Growth of guinea-pigs on fresh raw cabbage, oats, bran and water.

——— Curve A. Represents the standard growth curve of 3 animals upon a 30 g. fresh cabbage ration, oats, bran and water (Expt. 1, Tables II and VII).
— • — ,, B. Represents the average growth of 4 animals upon 15 g. fresh cabbage ration, oats, bran and water (Expt. 2, Tables II and VII).
• • • • • ,, C. Represents the average growth of 3 animals upon a 5 g. fresh cabbage ration, oats, bran and water (Expt. 3, Tables II and VII).

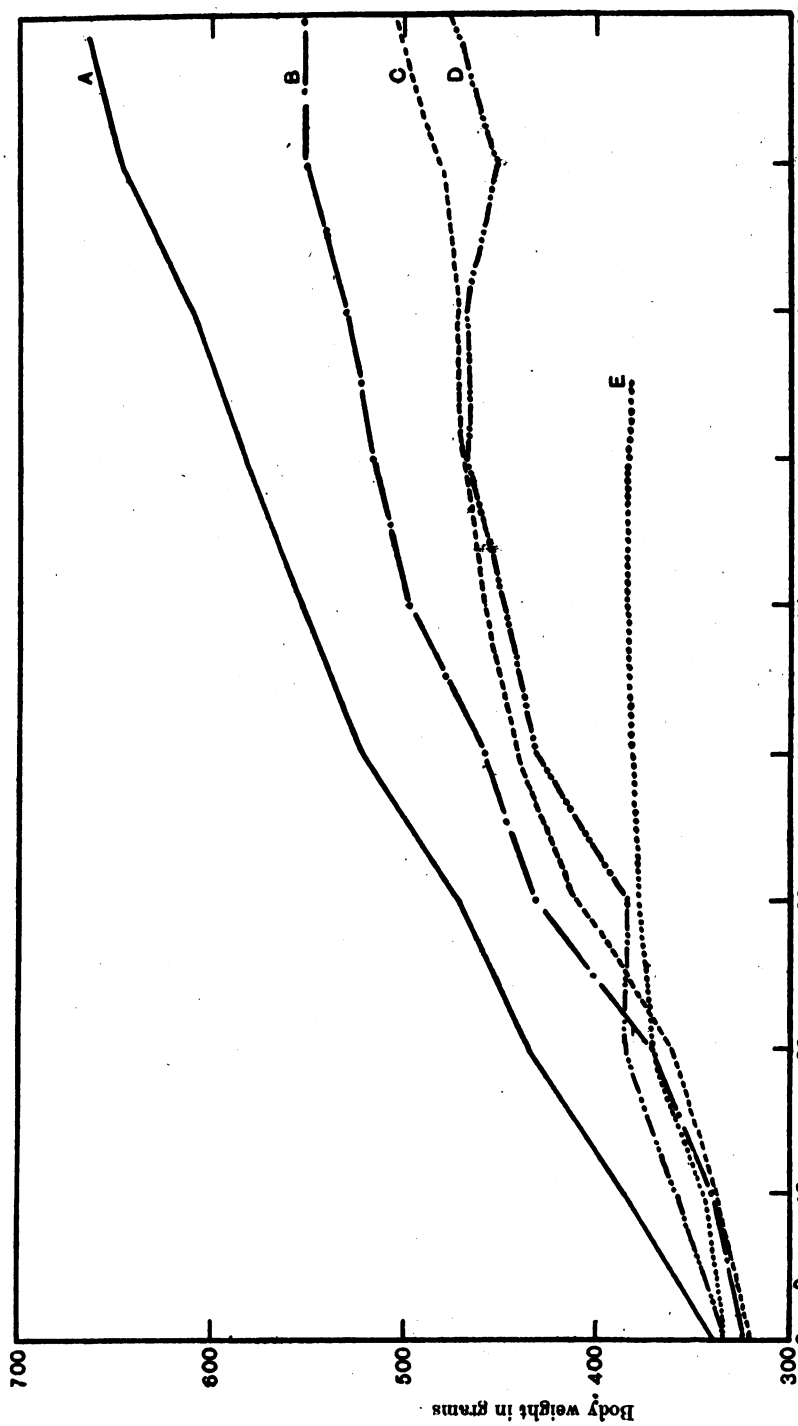

Fig. 2. Growth of guinea-pigs on fresh raw cabbage, oats, bran and autoclaved milk.

Mean weight curves of five groups of young guinea-pigs upon a diet of oats, bran, fresh cabbage and autoclaved milk daily. Each curve is obtained from the mean weights of all the animals in the experiment after definite time intervals (Tables II, VII, Expts. 1 and 4–7).

——— Curve A. Represents the standard growth curve of 8 animals upon a 80 g. cabbage ration (Expt. 1, Tables II and VII).

—·— ,, B. Represents the average growth curve of 4 animals upon a 5 g. cabbage ration with 60 cc. autoclaved milk.

– – – ,, C. ,, ,, ,, 4 ,, 2·5 g. ,, ,, ,,

—··— ,, D. ,, ,, ,, 4 ,, 1·5 g. ,, ,, ,,

····· ,, E. ,, ,, ,, 4 ,, 0·5 g. ,, ,, ,,

From this series of experiments we may therefore conclude:

(1) That normal growth on a ration of 30 g. fresh cabbage under the conditions of our experiments involves an increase of body-weight of about 100 per cent. in 90 days.

(2) That smaller rations involve a corresponding diminution in the growth rate without however causing any definite symptoms of scurvy to appear during life unless the ration is reduced to 0·5 g.

(3) That apparently satisfactory growth and health are obtained with rations from 1·5–5 g., if 60 cc. autoclaved milk daily is added to the diet, but suggestions of soreness during life, together with departure from the normal in the histology of the bone cartilage junctions point to a condition of incipient scurvy.

(4) That on a 0·5 g. ration well-marked symptoms of scurvy may be expected.

(5) That a 1 g. ration of fresh cabbage may be regarded as the minimum for protection from the symptoms usually diagnosed as scurvy.

The Antiscorbutic Value of Cooked Cabbage.

The only information available as to the effect of heat on the antiscorbutic properties of cabbage is contained in the paper of Holst and Frölich [1912] to which reference has already been made. Their general conclusion was that heat has a distinctly destructive effect and that this destruction is greater at 110° and 120° than at 100° for the same time of heating, viz. 30 minutes.

They used steam under pressure in an autoclave for heating the material at 110° and 120°, but at 100° the leaves were either boiled in slightly salted water or they were placed in an Erlenmeyer flask and heated for the required time, the leaves being slightly salted. The animals are said to have licked up the juice which exuded during heating, and also to have eaten their ration of cabbage: they appear to have refused to eat it when unsalted.

Table III gives a review of their results, the averages of the initial and final weights of the period of survival being calculated from their figures (H. and F., Table IV B, pp. 66–70). After being heated for ½–1 hr. at 100° a ration of 30 g. appears to have protected the animals almost completely from scurvy, but there was little or (in one set) no gain in weight as would have been expected on a corresponding ration of fresh cabbage. With 30 g. cabbage heated ½ hr. at 110° one animal out of three had clearly scorbutic symptoms (loose teeth, haemorrhages and disorganised rib-junctions) whilst all animals were similarly affected when the same ration was heated for 1 hr. at 120°.

TABLE III. *Summary of Experiments of Holst and Frölich with Cooked Cabbage.* [See H. and F., 1912, Table IV B, pp. 66–70.]

Diet	No. of animals	Mean time of life	Mean weight in g. Initial	Mean weight in g. Final	Result
30 g. cabbage cooked 1 hr. in ½ % common salt at 100° C. and oats	5	87 days	351	352	Complete protection except in one case
30 g. cabbage steamed ½ hr. at 100° and white bread	3	153	390	488	Complete protection
	3	105	353	293	Protection with loss in weight in two cases. Scurvy in one case
30 g. cabbage steamed ½ hr. at 110° and white bread	3	96	393	327	Scurvy, with partial protection
30 g. cabbage steamed ½ hr. at 120°	3	45	387	268	All with severe scurvy

In contradistinction to their results, our experiments with a 15 g. ration of cabbage autoclaved at 110°–130° for one hour gave almost complete protection from scurvy in each case (see Table V). This difference in result is difficult to account for, unless it be due to the different technique, to the use of green rather than white cabbage or to heating without the addition of salt. The present experiments are in accordance with one another although they differ in results from those of the Norwegian investigators.

In order to get some basis for comparison with the experiments in which small rations of fresh cabbage were employed, we selected 5 g. as the standard ration for investigating temperatures below 100°, giving the animals (as in experiments with raw cabbage) an allowance of 60 cc. autoclaved milk daily. In many cases the animals voluntarily drank their milk, which was given morning and afternoon, but in cases of failing appetite through the onset of scurvy, or other disinclination, hand feeding with a glass syringe was adopted (cp. fig. 6, Plate I). As with the fresh cabbage experiments, only the outer green leaves were used for heating. We found it unnecessary to use salt in cooking. Most animals accepted the cooked cabbage readily and the rest were hand fed.

At 60°, 70° and 80° entire leaves were plunged into water contained in a French cooker surrounded by water at constant temperature. The temperature was kept under control and was observed at intervals during the time of cooking, and pieces weighing 5 g. were cut off after the cooking.

For the experiments at 90° and 100° heating was done in steam. Small pieces were cut from a fresh leaf, each weighing 5 g.; these were suspended singly from hooks on the inside of a saucepan lid which just fitted the steam chamber over a saucepan of boiling water. By adjusting the rate of boiling

and also the steam outlet, the temperature of this chamber could be kept at 90° or 100° as required. In order to determine whether immersion in water at 100° itself caused any additional loss of antiscorbutic properties, a further set of animals were given a 5 g. ration taken from whole leaves which had been plunged into boiling water and boiled briskly for 20 minutes. They were then lightly drained, cut up and an amount equal to the 5 g. ration weighed out from them, an additional 0·5 g. being allowed to compensate for the extra water taken up in this method of cooking.

In all cases the cooking was done either daily or on alternate days, the cooked pieces being kept moist and placed in a refrigerator until wanted. The water content of the different samples was determined at intervals and varied from about 85–90 per cent., that of fresh cabbage varying from 87–90 per cent. of the fresh weight. The results of these experiments are summarised in Table IV and illustrated in the graphs, Fig. 3.

If the results are compared with those in Table II the following rough estimate of the degree of destruction of antiscorbutic material can be made. After one hour's heating at 60° the antiscurvy value of 5 g. cabbage is not greater than that of 1·5 raw cabbage, *i.e.* there is a loss of about 70 per cent. of the original value. Heating for the same time at 70° or 80° reduces the initial value of 5 g. raw cabbage to the equivalent of about 0·5 g. raw, *i.e.* a loss of 90 per cent. has taken place; after heating for one hour at 90° the loss exceeds 90 per cent.

From Table IV it is further evident that 5 g. cabbage heated either for one hour at 60° or for 20 minutes at 90°–100° has a similar effect in just protecting a young guinea-pig from scurvy. We have already seen that a daily ration of 1 g. fresh raw cabbage was the amount determined as necessary for protection from scorbutic symptoms (see p. 424). We may therefore regard 5 g. cabbage cooked for one hour at 60° as about equal in antiscorbutic value to 5 g. cabbage cooked at 100° for 20 minutes and to 1 g. raw cabbage. In other words the rate of destruction of antiscorbutic properties is increased about three times for a rise of temperature of 30° or 40°. If we assume that the destruction of antiscorbutic properties is uniformly accelerated by increase of temperature within this limit, the above results indicate the comparatively low temperature coefficient of about 1·3 for 10° rise of temperature. Assuming this value to be near the truth, then the effect of heating cabbage for one hour at 60° would be equivalent to heating for 46 minutes at 70°, or 35 minutes at 80°, or 28 minutes at 90°, or 21 minutes at 100°: and the experimental results show that the effect of heating 5 g. of cabbage for

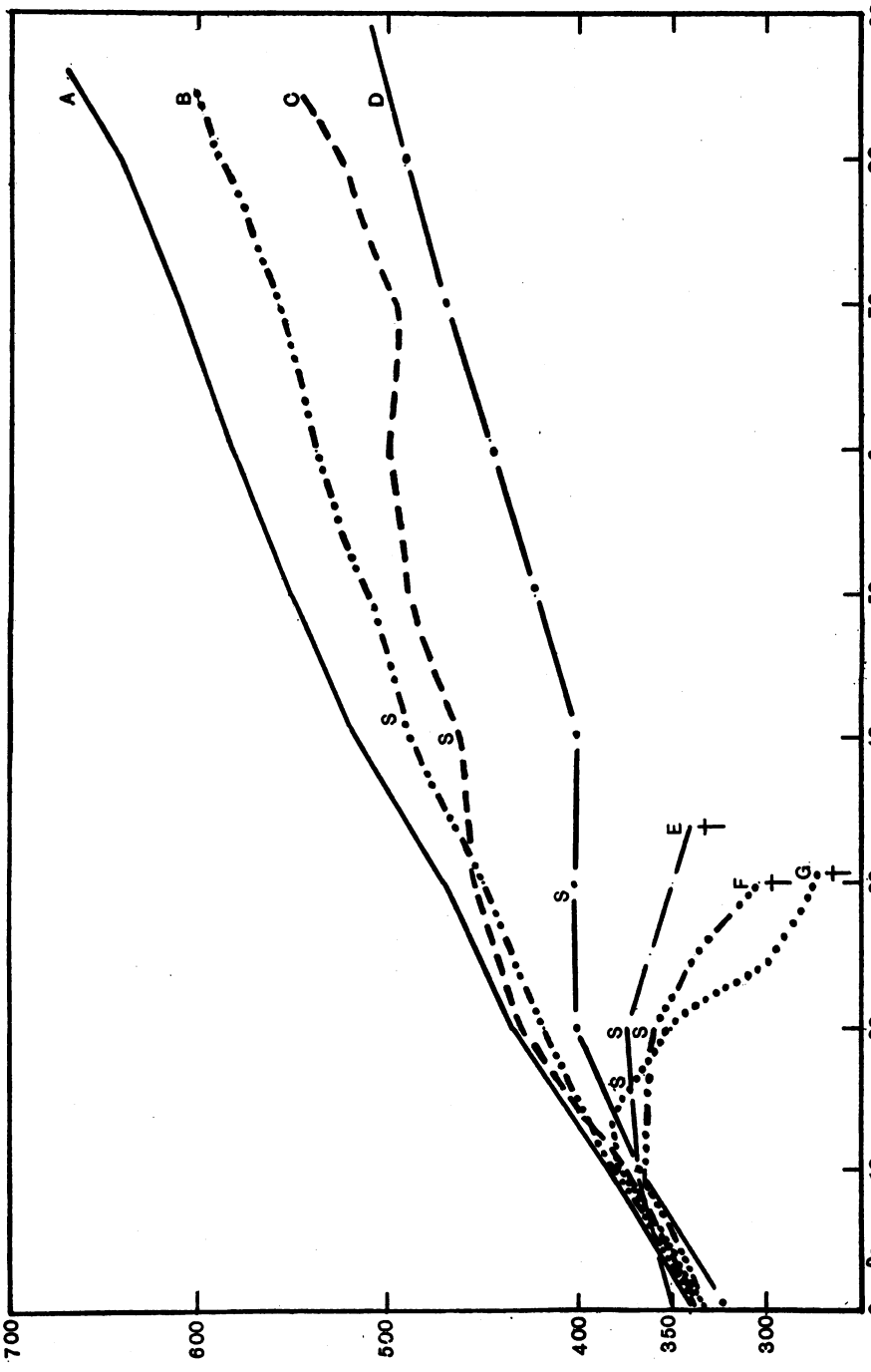

Fig. 3. Growth of young guinea-pigs upon a diet of fresh heated cabbage, oats, bran and autoclaved milk (Tables IV, VIII).
——— Curve A. Represents the standard growth of 3 animals upon 30 g. fresh cabbage, oats, bran and water (Expt. 1 Tables II and VII).
Curves B–F. Represent each the average growth of 4 animals upon heated cabbage, oats, bran and autoclaved milk.
-•-•-•- Curve B. Cabbage heated to 60° for 1 hour (Expt. 8). —•—•— Curve D. Cabbage heated to 100° for 20 mins. (Expt. 13).
— — — — ,, C. ,, ,, 70 ,, ,, (Expt. 9). — — — ,, E. ,, ,, 80 ,, 1 hour (Expt. 10).
—•••— ,, F. ,, ,, 90 ,, ,, At † an animal died and the average curve could not be continued (Expt. 11).
•••••• ,, G. Citric acid cabbage heated to 90° for 1 hour. At † an animal died and the average curve could not be continued (Expt. 11 A).
At S symptoms first appeared in one or more animals.

one hour at 60° is, in fact, about equal to heating for 20 minutes at 90°–100° (see Table II).

This comparatively small influence of rise in temperature, between the temperatures 60° and 100°, upon the destruction of the antiscurvy value of cabbage is of some interest in connection with the unknown nature of the antiscorbutic factor. It has been suggested that this substance might be a complex protein or an enzyme-like substance. The rate of destruction of most proteins by heat is greatly accelerated with rise of temperature. Egg albumin is coagulated about 635 times faster at 70° than at 60° [Chick and Martin, 1910]; the destruction of emulsin by heat has a temperature coefficient of 7·4 for the same range of temperature [Tamman, 1895], and the action of trypsin is accelerated 5·3 times for every 10° rise in temperature [Bayliss, 1908]. The comparatively insignificant effect of temperature-rise in accelerating the gradual destruction of the antiscurvy substance within the tissues of cabbage leaves, indicates that heat denaturation of a protein-like or of an enzyme-like body is not primarily involved.

In the plant, the antiscorbutic substance is produced and retained in the living cell. On heating at 100° or higher temperatures, killing is almost instantaneous, the collapsed cells becoming permeable alike to water and to the constituents of cell sap. At lower temperatures the killing is more gradual (about 10–15 minutes at 60°) and the tissues remain tough and leathery even after an hour's heating at this temperature. These differences in the behaviour of the tissues on heating may affect the rate of destruction of the antiscorbutic substance. Similar experiments with expressed juices of vegetables and fruits are in progress and may be expected to throw light on this point. According to Holst and Frölich, expressed juice of white cabbage loses nearly all its protective value after heating for 10 minutes at 60°, 70°, or 80°, the expressed juice being, in their opinion, much more thermolabile than when contained in the intact cells. We are at present unable to confirm this statement and in preliminary experiments with expressed juice of turnips a considerable amount of protective value was found to exist after heating for an hour at 80°.

Application of the foregoing Results to Methods of Cooking Vegetables.

The destruction of the antiscorbutic properties of fresh vegetables on heating has an evident bearing on their dietetic value when cooked or preserved. Cooking improves most vegetables by softening their tissues and increasing

TABLE IV. *Experiments with 5 g. rations Cooked Cabbage (+ 60 cc. autoclaved milk).* [For further details see Table VIII.]

Exp.	Cabbage ration: Temp. of heating	Cabbage ration: Time mins	No. of animals	Mean weight: Initial	Mean weight: Final	Total gain in wt. %	Duration of exp. in days	Symptoms during life	Post-mortem	Histology of rib-junctions	General conclusions
8	60° immersed	60	4	339	601	71	88–90	Soreness after 42–60 days	3 normal 1 slightly scorbutic	2 normal 2 incipient scurvy	Protection
9	70° immersed	60	4	334	519	55	90	2 showed no symptoms, 2 developed soreness 42–88 days, 1 showing also "scurvy position"	1 normal 3 fragile bones, and rib-junctions ridged 1 with slight muscular haemorrhage also	2 nearly normal ("incipient scurvy") 2 acute scurvy	Protection Partial protection
10	80° immersed	60	4	347	—	—	34-50, 1 case 90	Soreness after 28–32 days Soreness acute at first, but lessening "scurvy position" in 2 cases	Fragile bones rib-junctions nodular muscular and subcutaneous haemorrhages } in each case	1 nearly normal ("incipient scurvy") 3 acute scurvy	Protection in 1 case, slight protection in 3 cases
11	90° steamed	60	4	334	—	—	21–50	Soreness after 19–35 days, scurvy position, loss in weight	Fragile bones, rib junctions nodular haemorrhages severe	All acute scurvy	No protection
11 A	90° steamed, (citric acid cabbage)	60	4	337	270	−20	26–31	Soreness in 8–22 days	Acute symptoms	All acute scurvy	No protection
12	90° steamed	20	4	324	513	58	90	Soreness after 23–43 days No soreness in 1 case	All normal	All normal	Complete protection
13	100° steamed	20	4	329	485	47	90	3 with no symptoms 1 with slight persistent soreness after 27 days	do.	2 slight scurvy 2 normal	2 nearly protected 2 completely protected
14	100° immersed	20	4	327	493	50	3 lived for 90 (1 died in 30)	1 died in 30 days (pneumonia) with no symptoms: 1 lived 90 days —no symptoms: 2 developed soreness after 23 days	1 normal (after only 30 days) 3 with slight symptoms, brittle jaw, loose teeth, and in 1 case, ridged rib-junctions	1 normal 3 slight and 1 chronic scurvy	1 protected for 90 days 3 partially protected

their digestibility, but it certainly also causes some loss in their antiscorbutic value. This loss is the greater the longer the time of heating, and is very appreciable even when the temperature is far below the boiling point (*e.g.* 60°–70°). In general, slow cooking at a lower temperature is much more deleterious than more rapid cooking at a high one.

At the present time, owing to the shortage of fuel, there has been a great increase in the use of slow cooking of the hay-box type as a substitute for fire or gas cooking, both in private houses and in large communities, *e.g.* hospitals, factories, canteens, municipal kitchens. In this method of cooking, food is given 5–10 minutes' boiling on a stove, and then transferred to a closed box padded sufficiently to prevent much loss of heat by conduction. At least two hours is allowed in the hay box before the food is ready for consumption, and frequently four hours or longer is given, on the theory that in a hay-box it is impossible to over-cook the food. A well-made hay-box should only allow cooling to about 90° in two hours or to about 80° in four hours; but at these temperatures, as has been shown in the preceding section, the rate of destruction of the antiscorbutic factor is not much less than at 100°, although the softening of the tissues takes place much more slowly. It is clear therefore that the longer time needed in the hay-box is a great disadvantage in the case of green vegetables such as cabbage. So far as loss in antiscorbutic value is concerned, the effect of two hours in the hay-box is not much less than that of two hours' boiling.

In cooking green vegetables, it is customary to add a little soda to the water to preserve the green colour. It has been shown experimentally by other workers in this Institute [Harden and Zilva, 1918] that expressed orange juice loses its great antiscorbutic value when made even slightly alkaline shortly before administration. The destruction of the antiscurvy factor by weak alkalis would occur more rapidly at higher temperatures and would enhance the simple destructive effects of high temperature. At the present time, when vegetables form the chief source of antiscorbutic material in the diet of the great majority of the population, the use of soda in cooking should certainly be avoided.

The suggestion has been made[1], that, in order to lessen the inevitable loss of antiscorbutic properties suffered by vegetables on cooking, citric acid to the concentration of 0·5 per cent. should be added to the water in which they are boiled. This suggestion is based on the conclusion emphasised by Holst and Frölich, that fruit juices which are acid are more heat-stable in

[1] Private communication from Major E. D. W. Greig, I.M.S.

respect of the antiscorbutic factor, contained in them, than are the expressed juices of vegetables such as cabbage. According to these workers the juice of lemons and of raspberries retained its antiscurvy properties after heating to 100° for 60 minutes, whereas the freshly expressed juice of cabbage lost its antiscorbutic value after heating to 60°–100° for only 10 minutes. If however the expressed cabbage juice was acidified with 0·5 per cent. citric acid before heating some protection from scurvy was demonstrated. On the other hand expressed heated juice of dandelion leaves was no more protective when acidified, and sorrel leaf juice, which is acid in reaction when expressed, lost all its protective action when heated.

It therefore seemed worth while to investigate this point further and experiments were made on the influence of a weak solution of citric acid upon the antiscorbutic factor in cabbage leaves, both before and after heating. Four animals were fed on a 1·5 g. ration of raw cabbage leaves whose cut ends had 12–24 hours previously been placed in a 1 per cent. solution of citric acid; this passed readily up the vessels into the tissues of the leaf and could be demonstrated there with litmus paper. These four animals were given a ration of 60 cc. autoclaved milk daily and throve as well as those upon a 1·5 g. ration of raw cabbage without any citric acid (Exp. 6 A, Tables II and VII). When an acidified ration of 5 g. was heated to 90° for an hour, however, it had no longer any protective value. Four animals kept on a diet of oats and bran, 60 cc. autoclaved milk and 5 g. acidified heated cabbage died of acute scurvy in 22–30 days. Untreated cabbage heated for this length of time at 90° gave very slight protection, four animals on this ration dying of scurvy in 21–50 days. The addition of the acid therefore had certainly not improved the resistance of the cabbage to the destructive effects of heat[1].

The conclusion therefore to be drawn from these results is that in cooking vegetables it is better to add neither acid nor alkali to the water in which they are boiled.

Influence of Exposure to Temperatures above 100° upon the Antiscorbutic Value of Cabbage.

In order to get evidence as to the effect of heating at higher temperatures it was necessary to use larger rations. With an allowance of 15–30 g. daily, satisfactory nutrition was obtained, without the introduction of autoclaved milk into the diet, even when the cabbage had been heated to 100°–130° for

[1] This result has been confirmed in case of germinated lentils; when boiled for 15 minutes in water containing 0·5 per cent. citric acid, the destruction of antiscorbutic properties was found to be distinctly greater than when boiled for the same period in pure water.

one hour. For example, growth curves of animals fed daily on 15 g. cabbage steamed at 100° for an hour compared favourably with the growth curves of similar animals fed on 15 g. raw cabbage (cf. curve C, fig. 4, with B, fig. 1). Two animals were paired after 60 days of this diet and produced healthy young while still upon this limited cabbage ration.

A summary of the results obtained on heating cabbage to temperatures from 100°–130° as regards loss of antiscorbutic properties is set out in Table V and full protocols of the individual experiments may be found in Table VIII. Curves showing the average growth are shown in Figs. 4 and 5. It will be seen that protection from scurvy was obtained with the 15 g. ration in all cases. Even after heating to 130° for an hour, the symptoms of scurvy in life were only slight and at post-mortem examination the signs consisted of slight ridges upon the rib-junctions in three cases out of four, and small muscle haemorrhages in two cases out of four. All animals in Exps. 15–21, Table V, were in good health after three months upon the specified diets. If, however, slighter manifestations of scurvy were carefully looked for, there is on the whole progressive increase in the degree to which these are apparent, as the temperature at which the rations are heated is raised from 100°–130°. This is indicated by the increase of the number showing slight abnormalities of the histology of the rib-junctions and by gradations from slight tenderness (Expts. 18, 19, cabbage heated at 110° and 120° respectively for one hour) to definite and permanent soreness and swelling of the joints (Expts. 21 and 22, where the cabbage was heated for one and for two hours respectively at 130°).

In Exp. 21 (cabbage heated at 130° for one hour) slight but definite muscular haemorrhages were found beneath the muscles of the knee in two out of four animals after 90 days upon this diet. But not even in this case can the animals be said to have developed well-marked scurvy, and it is clear that there is a large margin of protection against scurvy in the raw 15 g. ration. After heating to 130° for one hour this becomes reduced to the equivalent of rather less than 1·5 g. raw cabbage and greater than 0·5 g. raw cabbage (as may be deduced from a comparison of symptoms with those of Expts. 6 and 7, Table II), and may be estimated at approximately the equivalent of 1 g. raw cabbage. On this basis we arrive at the conclusion that on heating for one hour at 130°, 15 g. of cabbage loses $\frac{14}{15}$, or 93 per cent., of its original antiscurvy properties. In Exp. 19, cabbage heated for one hour at 120°, the five animals were protected from any marked symptoms of scurvy, and the residual antiscorbutic factor may be estimated as, at least, the equi-

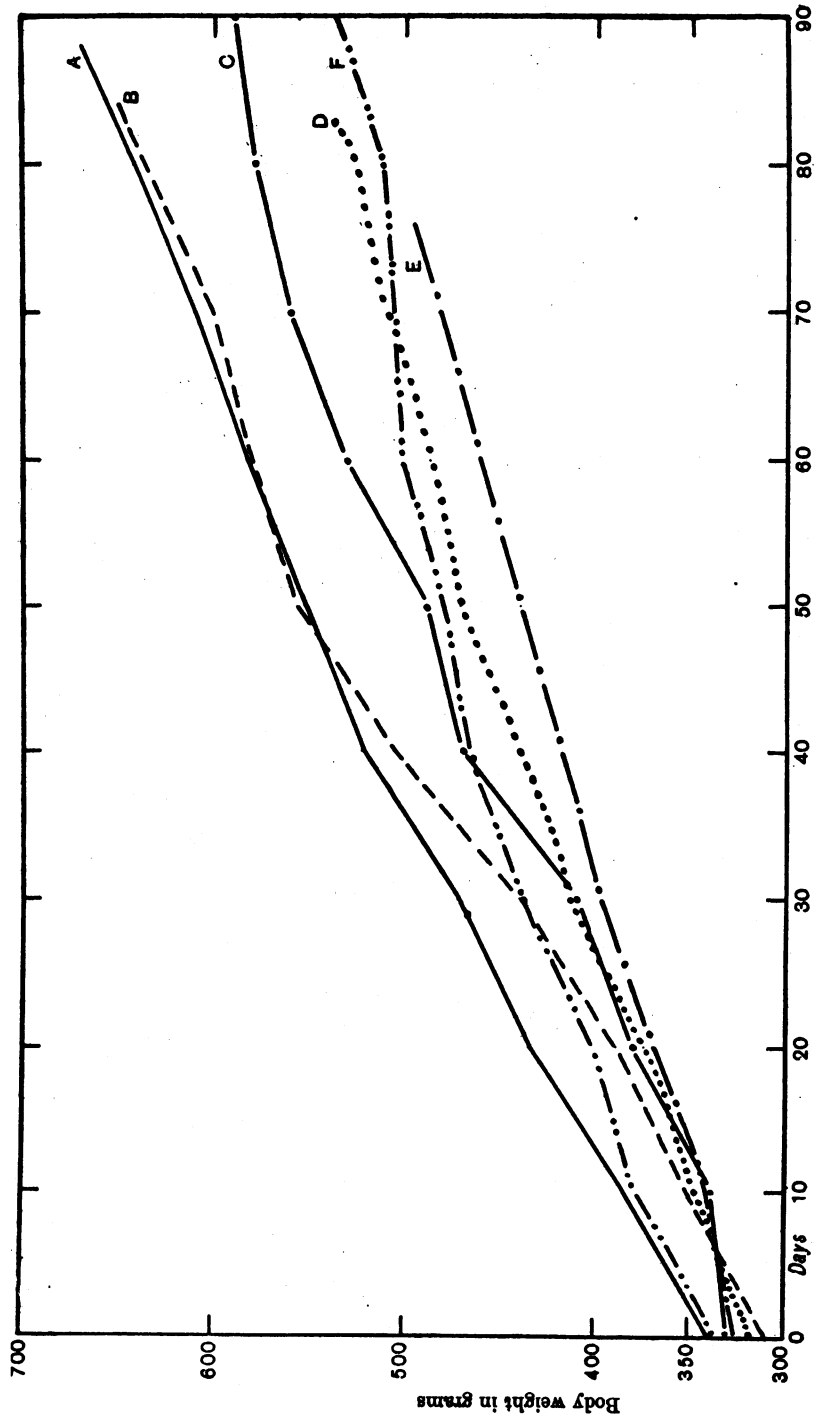

Fig. 4. Mean weight curves of young guinea-pigs on a diet of cabbage cooked 1 hour, oats and bran *ad lib.* and water. Each curve represents the mean weight of all the animals in one experiment calculated from time to time (Tables V and IX).

——— Curve A. Represents the standard growth curve of 3 animals on a 30 g. ration of raw cabbage (Expt. 1, Tables II and VII).

– – – – ,, B. Represents the mean growth curve of 3 animals on a 30 g. ration of cabbage cooked at 100° for 1 hour (Expt. 15).

——•—— ,, C. ,, ,, ,, 4 ,, 15 ,, ,, 100° ,, (Expt. 16).

• • • • • ,, D. ,, ,, ,, 4 ,, 15 ,, ,, 110° ,, (Expt. 18).

•—•—• ,, E. ,, ,, ,, 4 ,, 15 ,, ,, 120° ,, (Expt. 19).

—•—•— ,, F. ,, ,, ,, 4 ,, 15 ,, ,, 130° ,, (Expt. 21).

TABLE V. *Experiments with* 15 *g. cabbage + oats and bran + water.* [For details see Table IX.]

Exp.	Ration	Number of animals	Mean initial weight in g.	Total gain %	Time of exp. in days	Symptoms in life	Post-mortem examination	Histology of rib-junctions	General conclusions
15	30 g. cabbage steamed 1 hr. 100°	3	317	109	90	None	Normal	Normal	Complete protection
16	15 ,, ,, ,, 1 ,, ,,	3	327	82	90	do.	do.	do.	do. do.
17	15 ,, ,, ,, 2 ,, ,,	5	337	56	90	Temporary soreness in 1 case	Normal (fragile bones in 1 case)	do.	do. do.
18	15 ,, ,, ,, 1 ,, 110°	5	318	71	85–112	Slight soreness	Normal	Nearly normal or normal	Complete protection (nearly complete protection in 1 case)
19	15 ,, ,, ,, 1 ,, 120°	4	326	56	90	Sore knees in 1 case	Jaw fragile in 3 cases	Normal in 2 cases, nearly normal in 2 cases (incipient scurvy)	Complete protection in 2 cases, protection in 2 cases
20	15 ,, ,, ,, 2 ,, 120°	4	330	70	90	Soreness in all after 18–26 dys	Bones slightly brittle	3 normal 1 nearly normal	Complete protection in 3 cases, protection in 1 case
21	15 ,, ,, ,, 1 ,, 130°	4	346	55	90	Soreness in all after 15–28 dys	Nearly normal, slight haemorrhages in 2 cases	2 normal	2 complete protection
								2 incipient scurvy	2 protection
22	15 ,, ,, ,, 2 ,, 130°	3	343	12	88	Sore knees in 32 days	Bones fragile, teeth loose	2 definite scurvy	2 partial protection
								2 incipient scurvy	2 protection
	(with milk)	1	335	100	86				

valent of 1·5 g. raw cabbage, *i.e.* a loss has taken place not exceeding 90 per cent. When heating was continued for a second hour at this temperature the antiscorbutic value was still not reduced below that required for protection. On heating for two hours at 130°, however, definite scurvy symptoms in the form of swollen joints were apparent within 40 days. In this case the 15 g. ration of heated cabbage may be reckoned equivalent to something less than 1 g. but distinctly more than 0·5 g. raw cabbage in antiscurvy value and the loss suffered in this respect may be estimated at more than 93 per cent., and less than 97 per cent.

These rough estimates are collected in Table VI, and for purposes of comparison, the results of the preceding experiments in which the heating was done at temperatures between 60° and 100° are included. It is surprising to find that the destruction at the higher temperatures (Exps. 16–22) is not more complete. Even with the larger ration of 15 g., if the proportional destruction of antiscorbutic factor had proceeded on the same lines as at the temperatures below 100°, we should expect to find the residual amount reduced below the minimum required for protection (the equivalent of about 1 g. raw cabbage) at a temperature much lower than 130°, with one hour's heating. As a matter of fact, definite scorbutic symptoms occurred only when the *15 g. ration was heated for two hours at 130°.*

TABLE VI. *Showing approximately the degree of destruction sustained by the antiscorbutic and growth-promoting properties respectively, of fresh cabbage leaves when exposed to various temperatures.*

				Antiscurvy value		Growth promoting value	
Exp. no	Ration of cabbage, g.	Time of heating, minutes	Temperature C.°	Approximate estimate of heated material expressed in g. of raw cabbage	Loss expressed as % of the original	Approximate estimate of heated material expressed in g. of raw cabbage	Loss expressed as % of the original
8	5	60	60	1·5	70	—	—
9	5	60	70	0·5	90	—	—
10	5	60	80				
11	5	20	90	1·5	70	—	—
12	5	60	90	less than 0·5	more than 90	—	—
13	5	20	100	1·5	70	—	—
16	15	60	100	more than 1·5	less than 90	15	0
17	15	120	100	do.	do.	greater than 5	less than 66
18	15	60	110	do.	do.	—	—
19	15	60	120	do.	do.	—	—
20	15	120	120	more than 1	less than 93	—	—
21	15	60	130	1	93	5	66
22	15	120	130	less than 1 / greater than 0·5	more than 93 / less than 97	0	100

This point is shown perhaps even better by comparing the experiments in which the heating was done at 100° for both larger and smaller ration. Taking Exp. 13 as standard, with a loss of approximately 70 per cent. antiscurvy value after 20 minutes' heating at 100°, and applying this result to Exp. 16, we should expect the antiscurvy factor of the 15 g. ration heated for one hour at 100° to be reduced to the equivalent of 4·5 g. raw cabbage at the end of 20 minutes: to that of 1·4 g. raw cabbage at the end of 40 minutes: and to that of 0·42 g. raw cabbage at the end of 60 minutes. In other words the animals in Exp. 16 should have developed scurvy to a degree similar to that shown by the animals upon a 0·5 g. ration of raw cabbage, see Exp. 7, Table II. As a matter of fact they did nothing of the sort, but remained in excellent health throughout and were enabled to breed satisfactorily upon the diet.

It is therefore not possible to apply, by extrapolation, the results of experiments with a 5 g. cabbage ration to those in which a 15 g. ration is heated at higher temperature or for a longer time. There is no obvious explanation for this discrepancy, and we must conclude that in diets of this type, the *size* of the cabbage ration has some influence upon the degree to which its antiscurvy properties are destroyed by heat. It is evident that with the larger ration some extra source of protection from scurvy is being made available. In searching for this source, another point of difference in the two sets of experiments, is that the source of the fat-soluble growth factor is also different in the two cases. In case of the 5 g. ration, it is mainly derived from the allowance of autoclaved milk; in case of the 15 g. ration, it is derived wholly from the cabbage ration. It is not at once evident in what way this circumstance can affect the antiscorbutic reserves of the diet, and there is much evidence accumulated by the group of workers in this Institute, pointing to a distinct and separate action of these two factors in the nutrition of young guinea-pigs. Miss Tozer has, however, found that a lack of fat-soluble growth factor in the diet is accompanied by a series of changes in the histology of the rib-junction which is almost indistinguishable from that caused by a slight deficiency of antiscorbutic factor (see below, Appendix). It is therefore not impossible, that the antiscorbutic value of a diet may be enhanced and may show greater heat stability, when the antiscurvy factor and the growth fat-soluble factor are derived from the same food stuff. It is noteworthy that scorbutic symptoms supervened in animals fed with the 15 g. cabbage ration which had been heated to 130° for two hours, a point at which, as will be seen below, the reserves of the fat-soluble growth factor

were also exhausted. This factor is believed to be comparatively heat stable and the present investigation affords confirmatory evidence upon this point.

The suggestions contained in the preceding paragraph are put forward with reserve, especially as experiments are now in progress from which it is hoped that more light may be obtained. An investigation is being made on the influence of heat upon the antiscorbutic properties of expressed fruit and vegetable juices, which are deficient in the fat-soluble growth factor. This is supplied by a ration of autoclaved milk, and as the temperature of heating is raised the only change in the diet will be in the allowance of antiscorbutic material provided, the amount and source of the fat-soluble growth factor remaining unchanged throughout. This further investigation should also show whether the inclusion of the antiscorbutic factor in a living tissue has any influence upon its heat stability and the discussion of the whole subject may be profitably postponed until it is completed.

Influence of Heat upon the Growth-promoting Properties of Cabbage.

It has been shown that for full normal growth in a young guinea-pig on a cereal diet, a large daily ration of fresh cabbage (about 30 g.) is necessary. When this is reduced to 15 g. or further to 5 g. daily the resulting growth is also correspondingly diminished (see Table II and Fig. 1). There was no marked difference in appetite amongst the animals of the different experiments for the cereal part of the diet (Table VII), and the diminished growth can best be explained as caused by the lack of sufficient amount of some accessory growth factor in the diet. The work of McCollum and his colleagues, and of Osborne and Mendel has demonstrated that in the case of rats, two accessory growth factors are necessary in the diet if growth is to be made. In our experiments the water soluble growth factor was provided abundantly in the oats and bran ration *ad libitum* [McCollum and Davis, 1915, 1], whilst the fat-soluble growth factor shown to be widely distributed in green leaves [McCollum, Simmonds and Pitz, 1916] was strictly limited. It seems therefore that the limitation of the amount of growth on reducing the cabbage ration is due to the lack of fat-soluble A in the diet. This is in agreement with the general statement of McCollum and Davis that a small amount of the accessory substances is necessary for growth and that "above this amount growth appears to be in some measure proportional to the amount of the accessories present" [McCollum and Davis, 1915, 1]. When the 60 cc. ration

of autoclaved milk was added to the diets containing the small cabbage rations, this deficiency was repaired.

In the results of Expts. 3 and 4, Table II, a diet of 5 g. fresh cabbage with oats and bran and water can be compared with a similar diet to which 60 cc. daily of milk previously autoclaved at 120° for one hour has been added (cp. Fig. 1, curve *C*, and Fig. 2, curve *B*). The animals on the diet which included milk made much better growth than those without it, and their average weight-increase nearly equalled that of animals receiving 15 g. fresh raw cabbage daily and no milk. As regards growth, therefore, cabbage and autoclaved milk can replace each other. In the case of smaller rations of cabbage, *i.e.* of 2·5, 1·5 and especially of 0·5 g. cabbage, in spite of the consumption of the daily ration of autoclaved milk, growth never equalled that upon a 15 g. ration (Fig. 2). In these cases it appears that the reduction of the anti-scorbutic factor may itself have limited growth.

Both growth factors have been regarded as comparatively heat stable. Osborne and Mendel [1915] have shown that the growth promoting powers of butter fat remain unimpaired after heating for two hours at 100°. Other workers in this Institute have drawn attention to the growth promoting effects of milk which has been heated in an autoclave for an hour at 120° [Chick, Hume and Skelton, 1918]. More recently, Steenbock, Boutwell and Kent [1918] working with rats have demonstrated a destruction of the fat-soluble growth factor in butter fat after heating for four hours at 100°. These authors consider that the destructive process is one of low velocity which had not been revealed in the work of other investigators owing to the high initial content of the factor in the original material. These observations do not give any direct evidence as to the rate of destruction of the growth promoting substance, since no systematic comparison of the effects of heated rations has been made with a minimal raw standard ration.

By studying the growth curves of the animals fed on 15 g. cabbage rations heated at various temperatures, it is possible to investigate the influence of temperature on the fat-soluble growth factor.

Comparing C, fig. 4, and B, fig. 1, it is clear that the effect of heating a 15 g. ration of cabbage for one hour at 100° is not accompanied by any change in the amount of growth which was made on this diet, although a 15 g. raw cabbage ration is itself limiting, in that it does not allow maximum growth. A comparison of the slopes of the graphs of Fig. 4 shows that there is a progressive slight decrease in growth rate (10–20 per cent.) as the temperature of heating is raised from 100° to 130°. This can best be explained as the result

TABLE VII. *Experiments with raw cabbage rations.*

Exp.	No.	Sex	Ration of cabbage, g.	Wt of animal, g. Initial	Wt of animal, g. Final	Gain % on total weights	Time of exp. in days	Symptoms	Post-mortem	Histology of rib junctions	Food consumed: Oats, bran, g.	Food consumed: Cabbage, g.	Food consumed: Aut. milk, cc.	General conclusions
1	351	♂♀♀♂♀♂	30+water	350	670	91	87	No symptoms	Not examined	Not examined	57	30	—	Complete protection
	352			340	717		92	do.	do.	do.	47	29	—	
	865			330	675		90	do.	do.	do.	41	21	—	do.
	885			340	615		83	do.	do.	do.	61	30	—	do.
2	353	♀♂♂♀♂♀	15+water	330	555	74	90	do.	do.	do.	40	15	—	do.
	359			350	580		90	do.	do.	do.	59	15	—	do.
	826			330	620		90	do.	do.	do.	53	15	—	do.
	823			370	650		90	do.	Normal	Normal	55	15	—	do.
3	421	♂	5+water	330	525	26	90	Soreness after 37 days, persistent	Slight haemorrhages; bones slightly brittle	Nearly normal (incipient scurvy)	55	5	—	Protection
	422			320	445		90	No soreness	Normal	do. do.	61	5	—	do.
	425	♂		318	274		90	do.	do.	do. do.	39	5	—	do.
4	420	♀	5+aut. milk	340	530	69	90	Soreness after 44 days, persistent	Slight haemorrhages at knee	do. do.	42	5	55	do.
	423	♀♂♀		300	550		90	No symptoms	do. do	do. do.	26	5	57	do.
	424			335	565		90	Soreness after 24 days, persistent	Normal	do. do.	39	5	52	do.
5	440	♀♂♀♂♀♂	2·5+aut. milk	320	530	49	90	No symptoms	do.	do. do.	60	2·5	55	do.
	441			302	430		90	Soreness after 26 days, lasting 20 days	do.	do. do.	35	2·5	42	do.
	443			350	440		88	No symptoms	Tibia brittle	do. do.	35	2·5	55	do.
	379			340	515		90	Soreness after 40 days, persistent	Normal	do. do.	56	2·5	45	do.
	380			305	498		90	No symptoms	do.	do. do.	54	2·5	46	do.
6	389	♀♂♀♂♀♂	1·5+aut. milk	365	502	49	90	Soreness after 35 days, lasting 23 days	do.	Normal	34	1·5	53	do.
	390			330	478		90	No symptoms	do.	do.	48	1·5	45	do.
	391			320	350		70	Loss of weight, no soreness	do.	Slight scurvy	38	1·5	49	do.
	392			355	520		90	Soreness after 38 days, persistent	Jaw fragile	Normal	45	1·5	53	Slight scurvy
	861			355	675		90	Soreness after 33 days, persistent	Normal	do.	46	1·5	59	Protection
	862			325	404		90	Soreness after 37 days, persistent	Femur and tibia brittle; ribs ridged; jaw fragile	Nearly normal (incipient scurvy)	24	1·5	52	do.
6A	831	♀	1·5 (citric acid) +aut. milk	325	538	70	91	Soreness after 50 days, lasting only 10 days	Normal	Nearly normal (incipient scurvy)	69	1.5	60	do.
	832	♀		372	535		90	Soreness after 50 days, persistent	Jaw fragile, slight haemorrhages	do. do.	28	1·5	60	do.
	833	♂		300	615		90	Soreness after 49 days, lasting 34 days	Normal	Normal	63	1·5	60	Complete protection
	834	♂		322	565		90	Soreness, doubtful throughout	Rib junctions slightly ridged	Nearly normal	34	1·5	59	Protection
7	840	♂	0·5+aut. milk	335	427	18	90	Soreness after 79 days, persistent	Tibia brittle; 5 ribs ridged; slight haemorrhages	Normal	28	0·5	60	do.
	841			330	403		90	Soreness after 30 days, persistent Scurvy position	Tibia and femur brittle; 7 ribs ridged; teeth brittle	Chronic scurvy	25	0·5	58	Chronic scurvy
	847			328	275		67	Swollen knees; loss in weight	Tibia and femur fragile; all ribs ridged; jaw fragile; haemorrhages	Acute scurvy	15	0·5	38	Acute scurvy
	848			330	460		90	Soreness and swollen knees after 26 days, persistent	Four ribs ridged	Slight scurvy	24	0·5	60	Slight scurvy

TABLE VIII. *Experiments with 5 g. heated cabbage rations.*

Exp.	Ration of cabbage, g.	Time of heating, mins.	Temperature °C.	No. of animal	Mean weight of animals, g.		Gain in wt %	Time of exp. in days	Symptoms during life	Results of post-mortem examination	Results of histological examination	Food consumed, g.			General result
					Initial	Final						Oats, bran	Ant. milk	Cabbage	
8	5	60	60	866 ♂	355	615		86	Soreness after 42 dys	Normal	Nearly normal (incipient scurvy)	24	59	5	Protection
				867 ♀	320	568	71	90	,, ,, 42 ,,	3 rib-junctions ridged, lower molars brittle	do. do.	27	59	5	do.
				868 ♀	320	665		90	,, ,, 60 ,,	Normal	Normal	29	60	5	do.
				869 ♀	360	557		90	,, ,, 43 ,,	do.	do.	34	60	5	do.
9	5	60	70	835 ♀	325	437		91	No symptoms	do.	Nearly normal (incipient scurvy)	44	58	5	do.
				836 ♂	322	428	55	64	do.	Tibia fragile	do. do.	24	58	5	Protection (cause of death unknown)
				837 ♂	360	790		90	Soreness after 42 dys, scurvy position	3 rib-junctions ridged	Acute scurvy	43	60	5	Definite scurvy
				838 ♀	330	420		91	Soreness after 88 dys	Tibia and jaw fragile; 5 rib-junctions ridged; slight muscular haemorrhages	do.	25	56	5	do.
10	5	60	80	805 ♂	345	395		89	Soreness after 32 dys, with partial recovery	Jaw and teeth brittle; 5 rib-junctions ridged; haemorrhages in muscles	Nearly normal (incipient scurvy)	47	52	5	Partial protection (recovery from scurvy)
				806 ♀	355	356	−8	34	Soreness after 32 dys; scurvy position	All bones very fragile; severe muscular haemorrhages	Acute scurvy	24	52	5	No protection. Death from acute scurvy
				807 ♀	335	270		50	Soreness after 28 dys; scurvy position	do. do.	do.	41	41	5	Slight protection. Death from acute scurvy
				808 ♂	355	263		50	Soreness after 29 dys; scurvy position	do. do.	Definite scurvy	35	44	5	Slight protection. Death from acute scurvy
11	5	60	90 (steamed)	381 ♀	335	240		29	Soreness after 27 dys	do. do.	Acute scurvy	22	41	5	Death from acute scurvy
				382 ♂	350	280	−19	49	,, ,, 35 ,,	do. do.	do.	21	52	5	do. do.
				383 ♂	330	275		29	,, ,, 21 ,,	do. do.	do.	20	34	5	do. do.
				384 ♂	320	285		21	,, ,, 19 ,,	do. do.	do.	22	30	5	do. do.
11A	5 (citric acid)	60	90 (steamed)	393 ♀	330	255		26	,, ,, 17 ,,	do. do.	do.	26 (17 dys)	52	4·9 (22 dys)	do. do.
				394 ♂	355	270	−14	31	,, ,, 22 ,,	do. do.	do.	29	46	4·8	do. do.
				395 ♂	340	305		30	,, ,, 21 ,,	do. do.	do.	25	50	5	do. do.
				396 ♂	325	250		26	,, ,, 8 ,,	do. do.	do.	31	51	5	do. do.
12	5	20	90 (steamed)	801	340	510		90	Slight soreness after 30 dys, persistent	Normal	Normal	55	53	5	Complete protection
				802	320	458	58	90	Slight soreness after 43 dys, persistent	Nearly normal (jaw fragile)	do.	28	54	5	Protection
				803	320	575		90	Slight soreness after 23 dys (temporary)	Normal	do.	34	59	5	Complete protection
				804	315	508		90	No symptoms	do.	do.	36	58	5	do.
13	5	20	100 (steamed)	397 ♀	325	342		90	do.	do.	Definite scurvy (chronic)	35	57	5	Partial protection (chronic scurvy)
				398 ♀	338	520	48	90	do.	do.	Normal	67	58	5	Complete protection
				399 ♀	330	530		90	do.	do.	Definite scurvy (chronic)	27	49	5	Partial protection (chronic scurvy)
				399a ♂	325	560		90	Slight soreness after 27 dys, persistent	do.	Normal	41	56	5	Protection
14	5	20	100 (immersed)	385 ♀	330	238		28	No symptoms	Emaciated; muscular and subcutaneous haemorrhages	Definite scurvy	33	30	5	Partial protection (died of pneumonia)
				386 ♂	330	578	31	90	do.	Jaw fragile; 2 rib-junctions ridged	Nearly normal (incipient scurvy)	49	55	7	Protection
				387 ♀	320	360		90	Soreness after 23 dys, acute, but not persistent	Jaw fragile; teeth loose	Acute scurvy (chronic)	29	55	5	Protection (chronic scurvy)
				388 ♀	332	540		90	Soreness after 23 dys, persistent	Jaw fragile	Normal	42	46	5	Protection

TABLE IX. *Experiments with* 15 *g. heated cabbage rations.*

Exp.	Cabbage ration, g.	Heated		Animal		Wt of animals, g.		Duration of exp. in days	Mean gain %	Symptoms	Post-mortem	Histology of rib-junctions	Food eaten, g.		General conclusion
		Temp. °C.	Time, hours	No.	Sex	Initial	Final						Oats, bran	Cabbage	
15	30	100	1	333	♂	330	760	84	109	No symptoms	Normal	Normal	41	30	Complete protection
				334	♂	305	620	84		do.	Not examined	Not examined	47	30	,, ,,
				335	♂	315	610	84		do.	do.	do.	37	25	,, ,,
16	15	100	1	338	♂	300	598	90	82	do.	Normal	Normal	45	15	,, ,,
				339	♀	330	540	90		do.	Not examined	Not examined	40	15	Produced 1 young after experiment
				340	♂	350	645	90		do.	do.	do.	44	15	
17	15	100	2	354	♂	330	553	90	56	Soreness, temporarily, after 36 days' experiment	Normal	Normal	45	15	Complete protection
				355	♀	330	505	90		No symptoms	Not examined	Not examined	32	15	Two young; one born dead
				356	♂	355	465	90		do.	Muscles dark in colour	Normal	41	15	Protection
				357	♂	340	530	90		do.	Tibiae brittle; jaw fragile	do.	39	15	,,
				358	♂	330	560	90		do.	Jaw fragile	do.	35	15	,,
18	15	110	1	365	♂	325	602	90	71	Temporary soreness for 3 wks after 24th day	Not examined	Not examined	41	15	,,
				366	♂	337	660	90		No symptoms	do.	do.	43	15	,,
				367	♀	308	455	86		Soreness, persistent, after 63 dys	Normal	Normal	36	15	,,
				368	♂	296	505	85–112		Soreness, persistent, after 70 dys	do.	do.	46	15	,,
				369	♀	325	498	90		No soreness	Not examined	Not examined	50	15	,,
19	15	120	1	374	♀	315	510	90	56 (excluding No. 378)	Soreness after 56 dys, persistent	Muscles flushed	Nearly normal (incipient scurvy)	45	15	,,
				375	♂	345	517	90		No symptoms	Normal	Normal	37	15	,,
				376	♂	308	507	90		do.	Jaw fragile	do.	47	15	,,
				377	♂	335	515	90		do.	Not examined	Not examined	46	15	,,
				378	♂	335	456	46		Soreness after 16 dys, persistent	Slight muscular haemorrhage	Normal	37	15	,,
20	15	120	2	853	♀	325	622	90	70	,, ,, 33 ,, ,,	Normal	do.	44	15	,,
				854	♀	322	532	87		,, ,, 18 ,, ,,	Two rib-junctions ridged; severe visceral haemorrhage	do.	41	15	,,
				855	♀	325	310	35		Died of diarrhoea	Jaw and tibiae brittle	Nearly normal (incipient scurvy)	28	15	,,
				856	♀	345	515	?		Soreness after 35 dys, persistent	Slight haemorrhages beneath each knee	Normal	44	15	,,
				870	♀	330	575	89		,, ,, 56 ,, ,,		do.	44	15	,,
21	15	130	1	881	♂	349	656	91	55	,, ,, 15 ,, ,, scurvy position	Slight haemorrhages beneath each knee	Normal	47	15	,,
				882	♂	330	515	90		Soreness after 16 dys, persistent, 64 dys, faceache position	5 rib-junctions slightly ridged	do.	38	15	,,
				883	♀	348	522	91		Soreness after 27 dys, persistent	Slight haemorrhages beneath knees. All rib-junctions ridged, 3 with nodules	Nearly normal (incipient scurvy)	44	15	,,
				884		358	450	89		Soreness after 28 dys, persistent, but improving later	Rib-junctions narrowly ridged; molars loose	do. do.	29	15	,,
22	15	130	2	911	♀	330	315	90	12	Soreness after 31 dys, swollen knees	Tibiae brittle, ribs papery, jaw and teeth brittle	Definite scurvy	27	15	Partial protection
				912	♂	350	395	88		Soreness after 34 dys, slightly swollen knees	Ribs ridged, teeth brittle and loose	,, ,,	28	15	,, ,,
				913	♀	350	443	88		Soreness after 20 dys, right knee sore, becoming swollen	Slight haemorrhages	Incipient scurvy	30	15	Protection
				914*	♂	335	670	88	100	Soreness after 31 dys, knees slightly sore, becoming swollen	Knees swollen, molars loose	,, ,,	34	15	,,

* Note 914 received an average ration of 55 cc. aut. milk daily after the 33rd day.

of the destruction of some part of the fat-soluble factor during heating, seeing that the animals were otherwise in good health and there were no definite symptoms of scurvy. This destruction is increased with increase of time (Fig. 5) and at 130° there is a sharp contrast between the mean growth curve of animals fed on the ration heated for one and two hours respectively, the animals on the ration heated for two hours making practically no growth, although remaining very active. One of these animals was given a ration of 60 cc. autoclaved milk daily after the 30th day, and its weight at once rose 66 g. in the next 14 days, and was doubled by the end of the experiment (Table IX), showing that the increase of the fat-soluble factor in the diet caused an immediate access of growth.

By comparing the slopes of the average curves (Figs. 1–4) some idea of the rate of destruction of the fat-soluble growth factor in cabbage leaves at 100°–130° may be obtained. Curves representing the mean growth of experimental animals kept for 90 days on a 15 g. ration given raw (B, Fig. 1) and heated one hour at 100° (C, Fig. 4) are nearly identical. Cooking for an hour has therefore left the growth promoting powers but little impaired. When heating is continued at this temperature for two hours, the resulting growth is much less than that on a 15 g. raw ration but greater than that on a 5 g. raw ration in absence of milk, *i.e.* about half the original amount of growth factor has apparently been destroyed. The mean growth on a ration of 15 g. cabbage heated to 130° for one hour is approximately the same as that of animals on a 5 g. raw cabbage ration; this would indicate a destruction of 60–70 per cent. of the growth factor originally present. After two hours' heating at 130° little or no accessory growth factor remains. On the whole therefore, the fat-soluble growth factor is less sensitive to heat than the antiscorbutic factor. These conclusions are summarised in Table VI.

Summary and General Conclusions.

1. It has been shown experimentally that a very small amount of raw cabbage daily is sufficient to protect from symptoms of scurvy, when added to a diet of grain and autoclaved milk. Histological evidence shows, however, that an apparently healthy animal may be in a condition of incipient scurvy as regards the structure of its growing bones. Possibly these animals might develop symptoms of the disease if kept under less favourable conditions.

2. By a similar method of experiment, determination has been made of the antiscurvy value of cabbage after exposure to various temperatures for

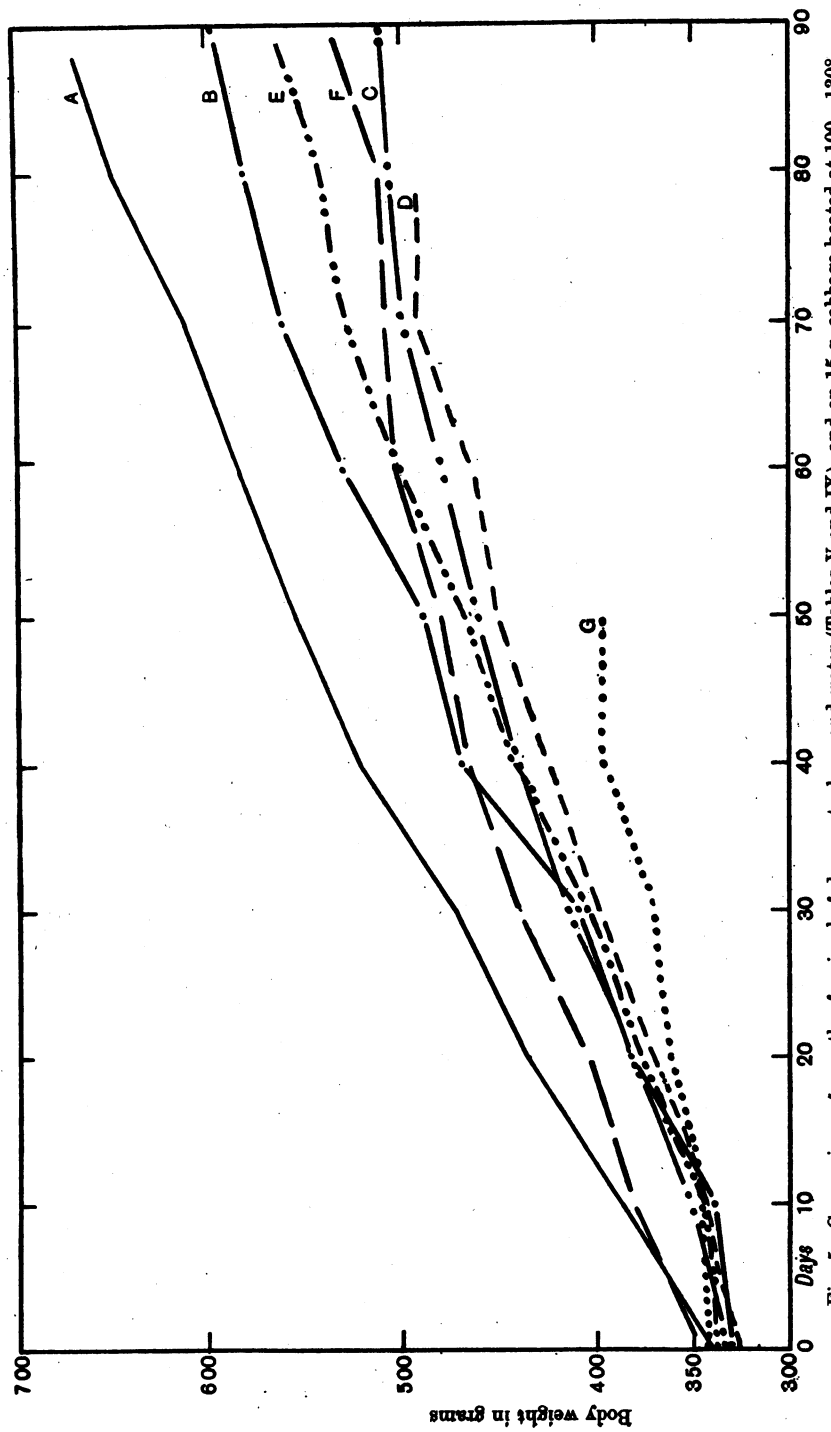

Fig. 5. Comparison of growth of animals fed on oats, bran and water (Tables V and IX), and on 15 g. cabbage heated at 100—130° for varying lengths of time.

——— Curve A. Represents the standard growth on 30 g. fresh raw cabbage, oats, bran and water (Expt. 1, Tables II and VII).
—·— ,, B. Represents the average growth of 4 animals on cabbage heated at 100° for 1 hour (Expt. 16).
—··— ,, C. ,, ,, 4 ,, ,, ,, 100° ,, 2 hrs. (,, 17).
— — — ,, D. ,, ,, 4 ,, ,, ,, 120° ,, 1 hour (,, 19).
—·—·— ,, E. ,, ,, 4 ,, ,, ,, 120° ,, 2 hrs. (,, 20).
—··—·— ,, F. ,, ,, 4 ,, ,, ,, 130° ,, 1 hour (,, 21).
····· ,, G. ,, ,, 3 ,, ,, ,, 130° ,, 2 hrs. (,, 22).

different intervals of time, and comparison made with that determined for raw cabbage. The antiscurvy factor was found to be exceedingly sensitive to temperatures below 100°. It is estimated that about 70 per cent. of the original value is lost after one hour's heating at 60° and over 90 per cent. after the same period at 90°. After 20 minutes' heating at 90° or 100°, the loss was estimated at about 70 per cent., *i.e.* the same as after 60 minutes at 60°.

3. It follows from the results detailed under (2) that the rate of destruction of the antiscorbutic accessory factor in cabbage leaves is increased only about threefold for an increase in temperature of 30°–40°. This low temperature coefficient is in opposition to the view that heat-denaturation of a protein-like or enzyme-like body is here in question.

4. These facts have some bearing on methods of cooking green vegetables, and indicate broadly that the least loss of antiscorbutic properties will be obtained by cooking green vegetables for a short time at a higher temperature rather than for a longer time at a lower temperature. Other evidence is quoted, which suggests that vegetables should be steamed rather than boiled in water (cp. Expts. 13 and 14, Table IV) and, if boiled, should be boiled in water only, the addition of either acid (citric acid) or alkali (soda) to the water increasing the inevitable loss of antiscorbutic vitamines.

5. Experiments are also described in which autoclaved milk was omitted from the diet and in which larger rations of cabbage were given (15 to 30 g.), heated to temperatures from 100° to 130° for periods of one to two hours. The destruction of antiscurvy properties, though extensive, was found to be much less than was stated by Holst and Frölich and also less complete than would have been expected from the results of experiments in which a smaller ration and lower temperatures were employed. It is suggested that this apparent discrepancy may be in part explained by the fact that in this series of experiments both the antiscorbutic factor and the fat-soluble accessory growth factor are drawn from the same food stuff.

6. The results of experiments described in (5) throw some light on the heat stability of the fat-soluble growth factor present in green cabbage leaves. The effect of heating for 1–2 hours at temperatures from 100°–120° was slight though perceptible. After heating at 130° for two hours, however, a serious amount of destruction had taken place.

7. Further experiments which are in progress with expressed juices of fruits and vegetables given raw and after exposure to different temperatures are expected to throw more light upon the problems dealt with in (5) and (6).

I am indebted to Dr H. Chick of this Institute for much help in carrying out these experiments, and for valuable suggestion and criticism in their interpretation. I am also indebted to Miss O. Lodge, formerly an assistant in this Department, for her care of the animals used in Expts. 4, 5 and 6; to Miss D. Gardiner for much assistance in the care of many of the experimental animals. I have also to thank my brother, Mr T. F. Delf, for his skill and patience in obtaining the photographs of Plate I.

APPENDIX

By FRANCES MARY TOZER.

THE illustrations (Plate II) are diagrammatic drawings made from histological preparations of the rib-junctions of animals used by Dr Marion Delf in her series of experiments. They are intended to demonstrate the more important histological changes which are observed in animals suffering from scurvy in varying degrees.

The diagnosis made from the histological preparations is, in all cases, independent of Dr Delf's diagnosis of the condition seen during the life of the animal or at the post-mortem examination. The particulars of the experiments were not supplied until the microscope slide was made and reported on, thus eliminating any bias on the part of the observer.

The results as a rule tally closely.

The slight changes observed in the rib-junctions in cases diagnosed as "Nearly Normal" or "Incipient Scurvy" were found to correspond with general good health: in many cases growth was satisfactory, no marked symptoms of scurvy were detected during life and, with very few exceptions, departure from the normal appearance at the post-mortem examination, if any, was insignificant.

The condition found in cases described as "Definite Scurvy" was accompanied by definite symptoms of soreness and a sub-normal condition during life combined with scorbutic lesions on post-mortem examination. The more marked changes in "Acute" cases correspond with severe illness during life and usually death from the disease [see Holst, 1907; Holst and Frölich, 1912; Chick, Hume and Skelton, 1918, p. 137]. These latter authors describe the changes at the rib-junction in the following words:

"Typical changes take place in the costo-chondral junctions. They become swollen, and exhibit a transverse yellow bar.... These appearances correspond with the complete disorganisation of the bone-cartilage junction, and with disappearance of the cartilaginous trabeculae and of the rows of cartilaginous growing cells. In many cases the bone is found to be completely fractured and there is great proliferation of connective tissue across the junction."

This description applies to the "Acute" condition. "Chronic" cases are those which, though suffering from scurvy in varying degrees, are sufficiently protected to prevent death within a period of 90 days: they show, histologically, the condition seen in diagrams 5 and 6. In these cases it seems probable that the ossified band across the junction is an attempt to strengthen the junction in an abnormal manner, the normal process having broken down to an extent depending on the severity of the disease. It is therefore apparent that "Chronic" cases vary in severity according to the degree of protection given.

It is not intended to deal here with the histological changes in the bone cartilage junctions possibly caused by deficiency of the growth, or any other accessory food, factor. In case of the animals to which the following diagrams apply these factors were provided in the diet by oats and bran and autoclaved milk respectively, and it is believed that the amounts consumed were adequate. In other experiments however, where a liberal supply of an antiscorbutic was given and where the fat-soluble A growth factor was known to be deficient, the resulting histological changes in the rib-junctions of the animals examined were found to resemble closely those of "Definite" or of "Definite Chronic" scurvy. In these test cases the antiscorbutic chosen was one believed to be deficient in the fat-soluble A growth factor (for example orange juice). This fact is mentioned because in the case of an animal not receiving an adequate supply of fat-soluble A the resulting changes in the rib-junctions are not dissimilar from, and are likely to be confused with, those caused by scurvy alone.

Note added 13. 1. 19. Since this paper was written further evidence has been obtained, confirming the statement that histological conditions indistinguishable from those termed "incipient" and "definite" scurvy may be caused by an insufficiency in the diet of the fat-soluble growth factor. In cases where the diet consisted of oats, bran and water with a small cabbage ration, it is possible that the histological findings should be thus interpreted (e.g. Table II, exp. 3). Where, however, the cabbage ration, raw or heated, was large (15–30 g, Table V, expts 19 and 21) or where milk was added to the diet (e.g. Table II, expts 5 and 6, Table IV, exp. 8), we have attributed these changes to slight deficiency in the antiscorbutic factor.

Some doubt has, however, arisen as to whether the standard ration of 60 c.c. autoclaved milk may be considered an adequate source of fat-soluble growth factor for guinea-pigs in *all* cases. Further experiments, now in progress, are designed to determine with greater exactness the average amount of fat-soluble growth factor as autoclaved milk necessary for normal growth of guinea-pigs, when all other accessory factors are present in excess.

DESCRIPTION OF DIAGRAMS, PLATE II.

1. *Diagram of Normal Rib-Junction* as represented by guinea-pig 823 and others.
2. *Diagram of Rib-Junction to illustrate "Incipient Scurvy,"* as represented by guinea-pigs 420–424, 379, 380, 826. This rib-junction is very nearly normal, but shows what may be regarded as the first recognisable signs of the onset of the disease; these are:
   (*a*) An appearance of general abnormality (when compared with the normal).
   (*b*) Unevenness of the junction and slight disorganisation of the rows of cartilage cells.
   (*c*) Shortening of the length of the rows of cartilage cells.
   (*d*) Shortening, and decrease in number of the trabeculae.
   (*e*) Increased amount of blood in the marrow cavity.
3. *Diagram of Rib-Junction to illustrate "Definite Scurvy"* as represented by guinea-pigs 443, 848, 385, 391. Histological symptoms vary considerably in these cases (according to the severity of the disease), and may approach in appearance those characteristic of the "*Acute*" condition. The rows of cartilage cells may be almost normal, but are more often noticeably shortened and usually somewhat disorganised or arranged in circular groups. The trabeculae are usually about half the normal length and have a truncated appearance. The junction as a whole may present an uneven, slightly disorganised appearance and there is sometimes a certain amount of macroscopic deformity.
4. *Diagram of Rib-Junction to illustrate "Acute Scurvy"* as represented by guinea-pigs 381–384, 806–808, 847. In these cases the junction and rows of cartilage cells are often completely disorganised, causing a marked macroscopic deformity of the bone.

   The bone of the shaft is frequently fractured.

   The marrow is no longer in contact with the edge of the junction and the space is filled with a mass of connective tissue; this is very characteristic in cases of fracture. A condition in which the trabeculae have almost disappeared and the rows are much shortened is also found in "Acute Scurvy," in those ribs in which no fracture of the shaft has occurred; usually however there is little or no ossification across the junction.

   There is frequently haemorrhage into the marrow cavity.

Diagrams 5 and 6 illustrate types of "Chronic" scurvy.

5. *May be described as "Chronic Definite Scurvy"* as represented by guinea-pig 841.

   The rows are much shortened, but not disorganised and an ossified band extends across the junction.
6. *May be described as "Acute Chronic Scurvy"* as represented by guinea-pig 387.

   The junction is deformed, and the rows are very disorganised; the trabeculae have disappeared and an ossified band extends across the junction. There is no connective tissue to be seen and no existing, or recent, fracture.

   Particulars of the animals quoted by their numbers in this appendix are given in Tables VI–VIII.

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